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REVERSE OSMOSIS WATER PURIFICATION UNIT: EFFICACY OF CARTRIDGE FILTERS FOR REMOVAL OF BACTERIA AND PROTOZOAN CYSTS WHEN RO ELEMENTS ARE BYPASSED

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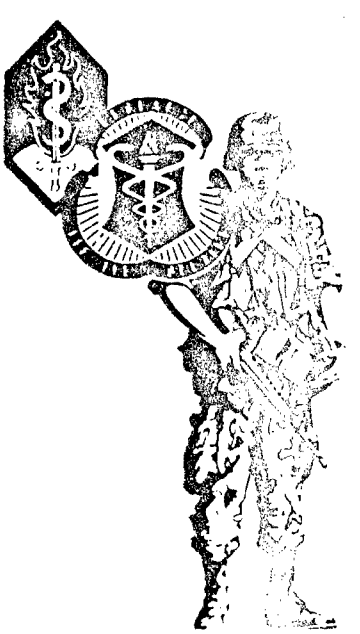

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Two different filter combinations have been tested as candidate systems for bypassing the reverse osmosis membranes of the Army's ROWPU when treating fresh water: a spiral-wound cotton prefilter of 5.0 µm nominal pore size combined with either a melt-blown polypropylene depth filter or a pleated polypropylene filter of 3.0 µm absolute pore size. Test organisms were Klebsiella terrigena, Cryptosporidium parvum oocysts, Rhodotorula rubra, and 3.7 µm latex beads. Challenge waters were dechlorinated tap water and a worst-case water containing AC fine test dust and humic acid. The depth filter, tested separately, achieved better than 99.9 percent reduction of C. parvum oocysts (the USEPA criterion) at filtration rates of 1-2 gpm under all conditions. The pleated filter did not achieve 99.9 percent reduction of C. parvum oocysts at a filtration rate of 1 gpm. None of the filter combinations tested was adequate for the removal of K. terrigena.

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PREFACE

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INTRODUCTION

The U.S. Air Force and Marine Corps have identified a requirement to bypass the reverse osmosis (RO) elements in the reverse osmosis water purification unit (ROWPU) for certain military applications. The RO membrane components of the ROWPU are provided as part of the treatment train so that drinking water can be produced from sea water, brackish water or chemically contaminated fresh water. Typically, the treatment of sea water yields 33 gallons of product water for every 100 gallons of feed water, while treatment of fresh water yields approximately twice as much. The Air Force and Marine Corps desire the capability to selectively bypass the RO elements when treating fresh source waters that meet chemical criteria for field drinking water, thereby recovering 100 percent of the treated water as product. An additional advantage of bypassing the RO membrane would be that source waters known to contain traces of chlorine or similarly corrosive chemicals could be treated without risk of damage to the RO membranes. This report describes a project conducted at the U.S. Army Biomedical Research and Development Laboratory (USABRDL) to evaluate ROWPU-compatible cartridge filters in terms of their ability to remove infectious protozoan cysts known to be resistant to military disinfection practices.

Tests were conducted using a 6-cartridge bench scale filtration apparatus constructed for the Naval Civil Engineering Laboratory (NCEL) by Separation Systems Technology, Inc. (SSTI).[1] Two of six candidate cartridge filters evaluated by SSTI for life cycle performance were selected by NCEL for microbial tests at USABRDL.

MATERIALS AND METHODS

CHALLENGE MICROORGANISMS. Microbiological methods used in these studies were in accord with the Guide Standard and Protocol for Testing Microbiological Water Purifiers.[2]

Bacterial Preparation. Klebsiella terrigena (ATCC 33257) stock cultures were grown for 24 hours at 36°C in nutrient broth to obtain a stationary phase culture. The culture was pelleted by centrifugation at 12,000 G, washed three times in sterile phosphate buffered saline (PBS) at pH 7.0, and filtered through sterile Whatman #2 paper to remove cell clumps. The stock bacterial suspension was diluted in PBS to contain ca. 1.0×10^8 colony forming units per mL (cfu/mL) using a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., Inc, NY). The K. terrigena suspension was quantified in triplicate by filtration of 1.0 mL volumes through 47 mm membrane filters (Type HAWG, 0.45 μm pore size, Millipore Corp., Bedford, MA). Each membrane filter was then placed on a 47 mm pad which contained 2.0 mL of m-Endo broth (Difco Laboratories, Detroit, MI) in a 50 x 9 mm snap-cap petri dish (Falcon 1006, Becton Dickinson & Co., Lincoln Park, NJ). Plates were incubated at 36°C for 24 hours, and the colonies displaying a green metallic sheen were counted.

Yeast Preparation. The morphological and size characteristics of Rhodotorula rubra (ATCC 36053) made the yeast suitable as a protozoan cyst simulant. The yeast cells were 3.5-4.5 μm in size with minimal budding (1.0 percent); they were readily distinguishable from indigenous yeasts by their pink color, and they could be quantified using membrane filtration techniques. Stock yeast cultures were prepared on YM agar (Difco) in 150-mm petri dishes. Each plate was inoculated with 1×10^5 yeast cells and grown at room temperature for 48 hours. Yeast cells from each plate were harvested and collected in 100-mL sterile, distilled deionized water, pooled and counted using a hemacytometer. These cells were further diluted to 2.0×10^7/mL in distilled deionized water containing 0.01 percent Tween 20™ [polyoxyethylene (20) sorbitan monolaurate (Aldrich, Milwaukee, WI), added to prevent clumping] and refrigerated. Yeast cells were determined to remain viable for at least 10-14 days when stored at 4°C; however, fresh stocks were grown weekly for use in these studies.

Cyst Simulant Preparation. For testing, 3.7 μm latex Accubeads™ (styrene divinylbenzene copolymer, Fastek) were diluted to a final concentration of 2.0×10^7/mL in sterile distilled deionized water containing 50 μg/mL of sodium dodecyl sulfate. Numbers of beads were quantified by means of hemacytometer using phase contrast microscopy.

Protozoan Oocyst Preparation. Infected calf feces (50 percent in 2.5 percent potassium dichromate) containing Cryptosporidium parvum oocysts were obtained from the University of Idaho and partially purified for the filter challenges using a modified method of Waldman et al.[3] The calf feces suspension was dispensed in 15-mL volumes into 50-mL conical polypropylene centrifuge tubes, and a volume of 25 mL of PBS (pH 7) containing Tween 20™ (0.05 percent), penicillin (100 units/mL) and streptomycin (100 μg/mL) was added to each tube. The tubes were mixed well (vortexed) and centrifuged at 750 G for 10 minutes; 30-mL volumes of the supernatants were discarded, and the pellets were resuspended in 25 mL of PBS. After the contents of the tubes were vortexed, all tubes were refrigerated overnight at 4°C. Next, each of the tubes was again mixed, sonicated for one minute and centrifuged at 750 G. A 25 mL volume of the supernatant was discarded from each tube and replaced with PBS containing 0.05 percent Tween 20™ but without antibiotics. The contents of each tube were again vortexed. Ten mL of anhydrous ether was added to each tube and mixed with the suspension for one minute. The tubes were then centrifuged at 500 G for 10 minutes, and the top three layers (ether, debris plug and PBS-Tween 20™) were removed and discarded. The pellets were resuspended in a final wash of PBS-Tween 20™ (0.01 percent) and centrifuged at 750 G. All supernatants were discarded, and the pellets containing the cysts were resuspended in PBS with 0.01 percent Tween 20™ and pooled. A sample of the oocyst suspension was diluted to 1:20 and 1:30, and the oocysts were counted in a hemacytometer using phase contrast microscopy. No morphological changes were observed in the oocysts which would indicate degradation had occurred by this purification process; the oocysts were spherical and measured 4.0-4.5 μm in size. The final stock suspension contained 5.42×10^9 oocysts/L. Phase-contrast microscopic examination of the cysts showed no morphological changes or losses due to excystation after storage at 5°C for 7 days. The partially purified oocyst suspension was determined, by fecal coliform analysis, to be free of interfering bacterial contaminants and, by microscopic examination, to be free of fecal debris and yeasts.

QUANTIFICATION OF CHALLENGE MICROORGANISMS AND SIMULANTS

Bacteria and Yeast Analyses. Test and control water samples containing K. terrigena were diluted in PBS and assayed on membrane filters in triplicate as described above using m-Endo broth. Colonies having a green metallic sheen were counted after 24 hours incubation at 36°C. R. rubra yeast samples in water were serially diluted in sterile distilled deionized water and quantified using the membrane filter method described above with YM broth at pH 3.3. The pink yeast colonies were enumerated after incubation at 36°C for 24 hours.

Oocysts and Bead Analysis. After challenge water samples were collected from each sampling point, final concentrations of 0.1 percent Tween 20™ and 1.0 percent newborn calf serum were added to each sample to prevent adsorption of the oocyst and bead materials to the glass collection flasks. Each sample was filtered through a 47-mm, 1.0 μm-pore size, polycarbonate membrane filter (Nuclepore Corp., Pleasanton, CA) followed by a wash of 100 mL of distilled deionized water containing 0.1 percent Tween 20.™ The membrane filter was transferred to a 60-mm plastic petri dish and washed with 5.0 mL of distilled deionized water containing 0.01 percent Tween 20.™ The wash was collected, transferred to a 50-mL polypropylene centrifuge tube and saved. The membrane was cut into eight pieces with a sterile scalpel and placed in a separate 50 mL centrifuge tube. Wash solution (10 mL) was used to rinse the petri dish, and that rinse was collected and used to wash the filter pieces in the second centrifuge tube. After the material in the tube was thoroughly mixed on a vortex mixer, the wash material was collected and pooled with the wash in the first tube; this step was repeated twice. The membrane was given a final 10-mL wash with vigorous mixing for one minute. The tube containing all the wash material was centrifuged at 1200 G for 10 minutes at 4°C. The supernatant was pipeted off and discarded, leaving a volume of 0.8-1.0 mL which was used to resuspend the pellet.

For quantification, a volume of 200 uL of a concentrated bead-oocyst suspension was pipeted into duplicate 4-mL polypropylene tubes. A volume of 100 μL of 2 percent malachite green was added to each tube and allowed to stand for 20 minutes at room temperature. A volume of 100 uL of 1 percent sulfuric acid was added to each tube just prior to counting. The tubes were vortexed and sonicated briefly to fully disperse the cycts and beads. A coverslip was placed on the hemacytometer, and the chamber was filled with sample using a pasteur pipet. The filled chamber was allowed to settle for a minimum of 2 minutes (beads settle slower than oocysts). For each sample tube one chamber of 5 squares was counted, unless the average of each square contained 1 or less; then two chambers or 10 squares were counted. Counts of duplicate sample tubes were averaged. If no beads were found in the diluted samples they were again counted using undiluted sample material. Beads appeared as well-defined, color-free opaque shiny spheres. A minimum detection level of 1.0×10^3 was established for counting the beads and oocysts using the hemacytometer under phase contrast microscopic conditions.

CHALLENGE WATER CHARACTERISTICS

General Challenge Water. Tapwater was collected in large fiber glass test tanks, and sodium thiosulfate (10 mg/L) was added to neutralize residual chlorine. The water was also stored overnight before testing to assure complete dechlorination..

Worst-Case Challenge Water. Tapwater was collected in large fiber glass test tanks and sodium thiosulfate (10 mg/L) was added to neutralize residual chlorine. Worst-case challenge water was prepared according to the Guide Standard and Protocol, Section 3.3.3, Test Water #3 (Challenge Test Water/Ceramic Candle or Units With or Without Silver Impregnation).[2]

(1) Turbidity: A turbidity of 30 nephelometric turbidity units (NTU) was obtained using 150 mg/L of AC fine test dust (A.C. Spark Plug Div., General Motors Corp., Flint, MI) for the challenge water as measured in a Model 2100A Turbidimeter (Hach Chemical Co., Ames IA). The test dust particle size distribution was follows:

Micrometers	Percent less than
5.5	38 ± 3
11	54 ± 3
22	71 ± 3
44	89 ± 3
176	100

(2) Total organic carbon (TOC): Humic acid, sodium salt (Cat. No. H1,675-2, Aldrich Chemical Co., Milwaukee, WI) was incorporated at 10 mg/L of test water.

(3) Temperature: Test water temperatures were ambient (ca. 20°C).

(4) Total dissolved solids (TDS): Sea salts (Cat. No. S-9883, Sigma Chemical Co., St. Louis, MO) were added at a concentration of 1500 mg/L of test water.

TEST FILTERS. Cotton prefilters used in these studies were 20-inch, spiral wound, 5.0-μm nominal pore size (DW 5-01-20-1, Delta Pure Filtration Corp., Ashland, VA, or the equivalent); six were placed in parallel in a pressure housing similar to that used on the 600-gph ROWPU. Single 10-inch prefilters of the same composition were used for preliminary studies. Two 10-inch cartridge filters, both rated at 3 μm pore size absolute, were evaluated for the RO bypass study. The Pall Profile (MCY1001Y030, since discontinued and replaced by RM1F030H21, Pall Corp., East Hills, NY) is a melt-blown polypropylene depth filter; the Nuclepore® polypropylene pleated filter (QMC-P-10"-0.5) has about 6.0 ft^2 effective filter area.

TEST STAND. Preliminary and prefilter studies were performed using a 10 inch filter cartridge holder connected to a feed supply tank and a collection tank. Pressures and flow rates were monitored and controlled by valves connected to pressure gauges installed immediately before the filter cartridge inlet and after the filter cartridge outlet. For studies simulating bypass of the ROWPU RO elements, the following system was provided by NCEL:

The test stand was 72 inches long and 36 inches wide, with a front panel height of 54 inches (Figure 1). The system was mounted on 8-inch semi-pneumatic wheels and was designed to accommodate six 10-inch cartridge filters. A 208-V single-phase centrifugal pump, protected by a 20-mesh strainer, provided feedwater at a maximum pressure of 90 psig. Feedwater pressure was regulated by a 0.5-inch PVC regulating valve prior to passing through the prefilter pressure housing described above. Filtered feedwater at approximately 13 gpm was reduced to a maximum of 80 psig and 12 gpm by a regulating valve at the system exit. Feedwater flow through each cartridge was maintained at 1.0-2.0 gpm by flow control valves. Instrumentation was provided to measure feedwater temperature, pressure, flow rate, cumulative feedwater flow, differential pressure across the prefilters and each cartridge filter, and flow rate through each filter. Differential pressure measurement across each cartridge was accomplished using a 7-round valve to allow switching from one position to another; a single differential pressure gauge was used with one of the valve positions connected to the feed manifold. Sampling valves allowed the collection of both raw and prefiltered feedwater during system operation.

RESULTS AND DISCUSSION

Preliminary Filter Characterization

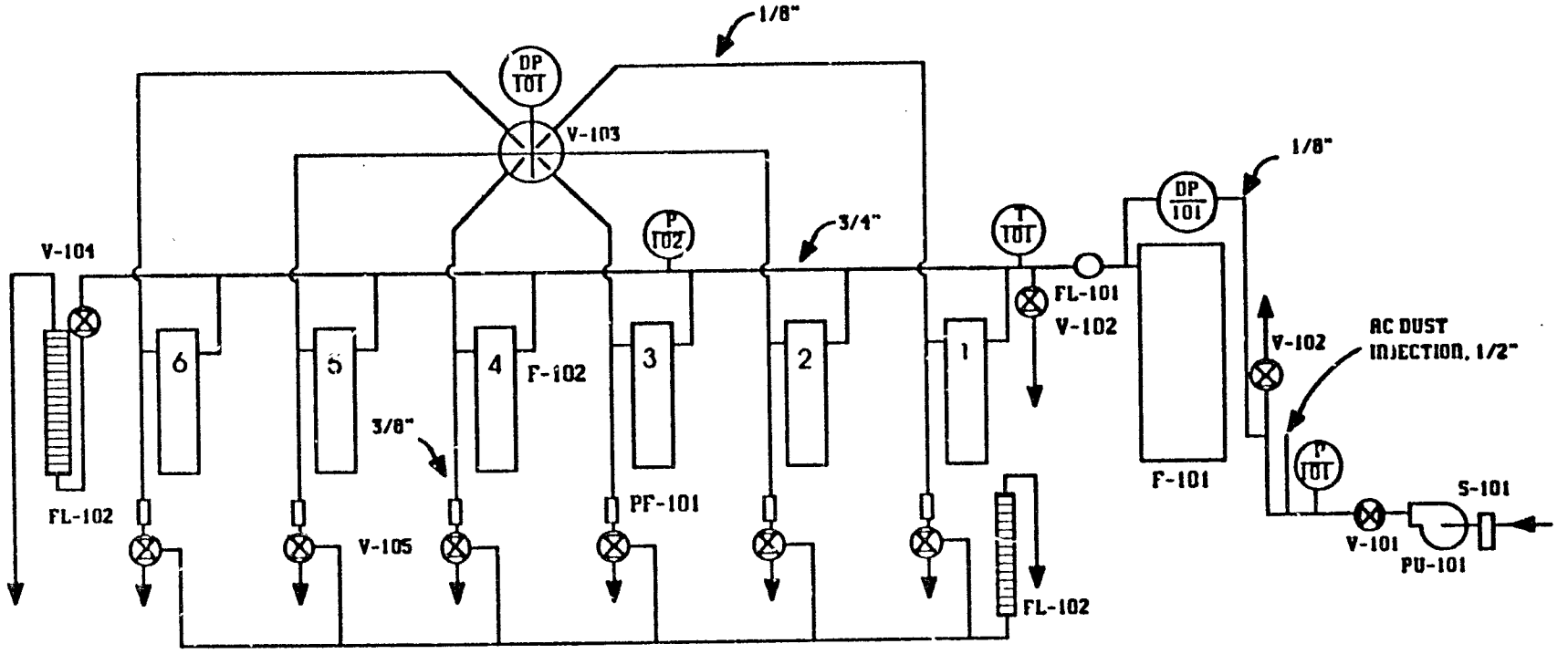
Two tests were run initially with the standard 5.0-μm ROWPU prefilters to establish changes in pressure and flow rates over time using AC test dust concentrations of 250 mg/L, corresponding to 57 NTU, and 50 mg/L in order to determine loading effects on the filters (Table 1). Initial flow rates were about 1 gpm. As would be expected, filter life, as measured by pressure differential elevation and associated flow rate reduction, was much longer at the lower turbidity loading (50 mg/L).

The initial tests were followed by a series of four tests on representative prefilters using 150 mg/L (30 NTU) of AC test dust, the level of challenge recommended in the Guide Standard and Protocol.[2] Prefilter efficiencies varied greatly with respect to pressure change (head loss) and flow rate for the four cartridges tested (Figures 2-4, Appendix Tables A1-A4). All runs were discontinued when the pressure differential reached about 20 psig, the typical cutoff for changing ROWPU prefilters in the field. Three filter runs continued to produce water at an acceptable rate for 360 or 390 minutes; in Run 2 the filter plugged after 120 minutes. The four filters tested generally provided product water with residual turbidity less than 1 NTU, although the initial time required to achieve this turbidity level varied with increased head loss.

SEPARATION SYSTEMS TECHNOLOGY

S-101 - In Line Strainer
PU-101 - Teel Pump, 14.5-13.3 gpm, 60-80 psi
P-101 - Pressure Gauge, 0-160 psi
V-101 - PVC Regulating Valve, 1/2"
V-102 - Brass Sampling Valve, 1/4"
V-103 - St. Steel 7-way Valve, 1/8"
V-104 - Brass Regulating Valve, 1/2"
V-105 - Brass 3-way Valve, 3/8"

F-101 - Prefilter Cartridges, 5 micron (6)
F-102 - Cartridge Filters
T-101 - Vapor Tension Thermometer, -40 to 50 C
P-102 - Pressure Gauge, 0-100 psi
DP-101 - Differential Pressure Gauge, 0-40 psi
PF-101 - Plastomatic Flow Control Valves, 2.0 gpm
FL-101 - Flow Totalizer, 1/4-20 gpm
FL-102 - Flowmeter, 0.3-3.5 gpm



ROWPU BYPASS FILTRATION TEST SYSTEM P&ID

TABLE 1. PREFILTER[a] PERFORMANCE, AC FINE TEST DUST ADDED

Time, min	250 mg/L AC test dust			50 mg/L AC test dust	
	Flow gpm	Head loss psi	Product turbidity NTU	Flow gpm	Head loss psi
0	0.90	2	24	[illegible]	2
30	0.71	4	4.5	[illegible]	2
60	0.63	14	1.2	[illegible]	3
90	0.48	24	0.94	[illegible]	3
120	0.34	28	0.55	0.90	3.5
150	0.26	31	0.56	0.90	4
180				0.87	5
210				0.82	10
240				0.77	13
270				0.66	16
300				0.61	19
330				0.58	22
360				0.50	24
390				0.48	26
420				0.45	27

a. Spiral wound cotton, 10 inch.

Another preliminary test to determine the cyst removal capability of the ROWPU prefilters was carried out by addition of latex bead simulant (2.0×10^6/L) and AC test dust (150 mg/L, 30 NTU) to 450 gallons of feed water. Samples were taken from the feed tank after 30 minutes and at the end of the run to detect any loss of beads due to electrostatic attraction to the tank or plumbing; no loss occurred. Filtered water samples were collected after 30, 75, 120, and 210 minutes, the last being the point in time where the filter clogged after passage of about 200 gal. Results are shown in Table 2. Latex bead removal improved to >99 percent as the filters became loaded with the test dust, while pressure differential increased and flow rate decreased.

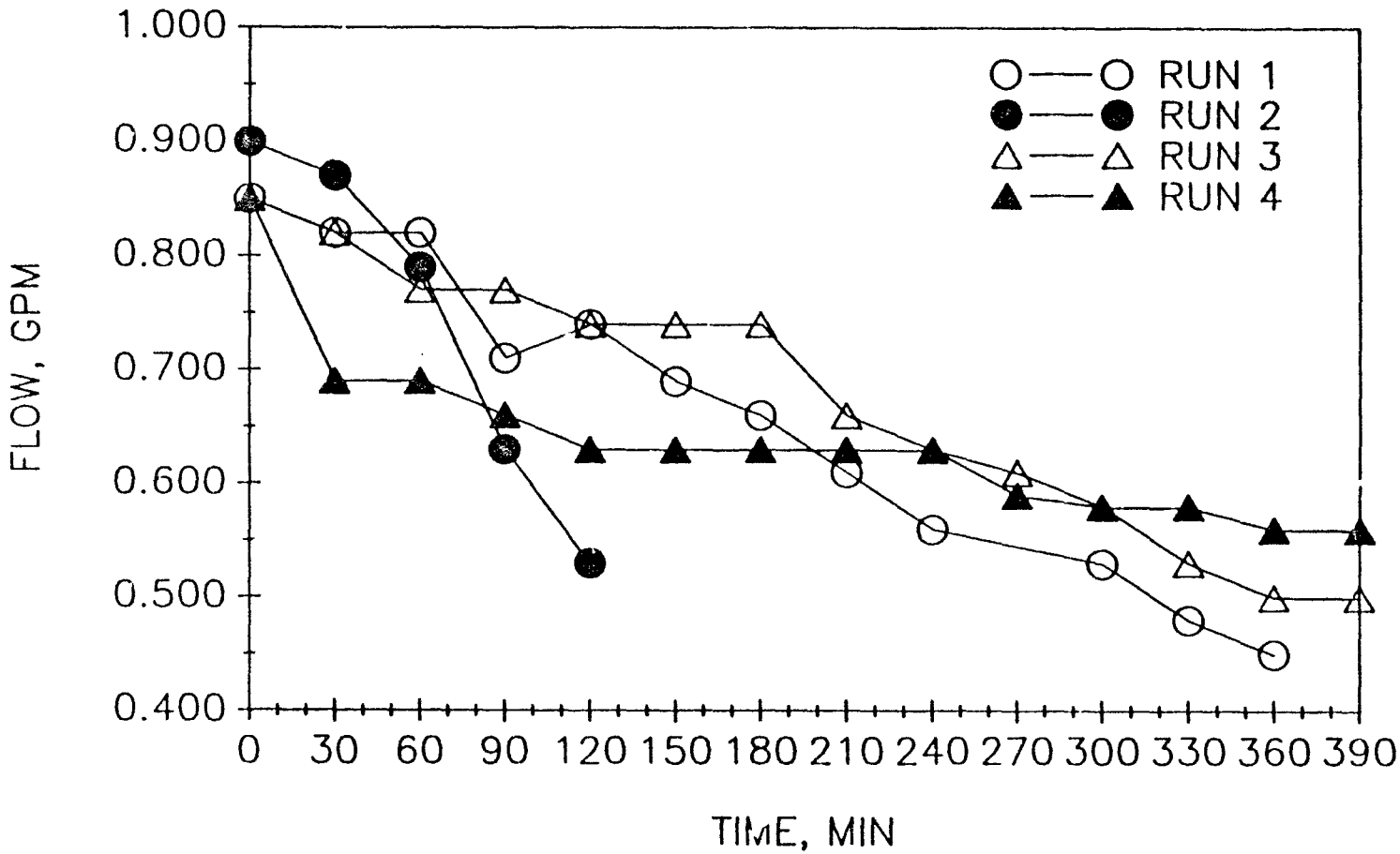


Figure 2. Prefilter performance, 150 mg/L fine test dust added: flow

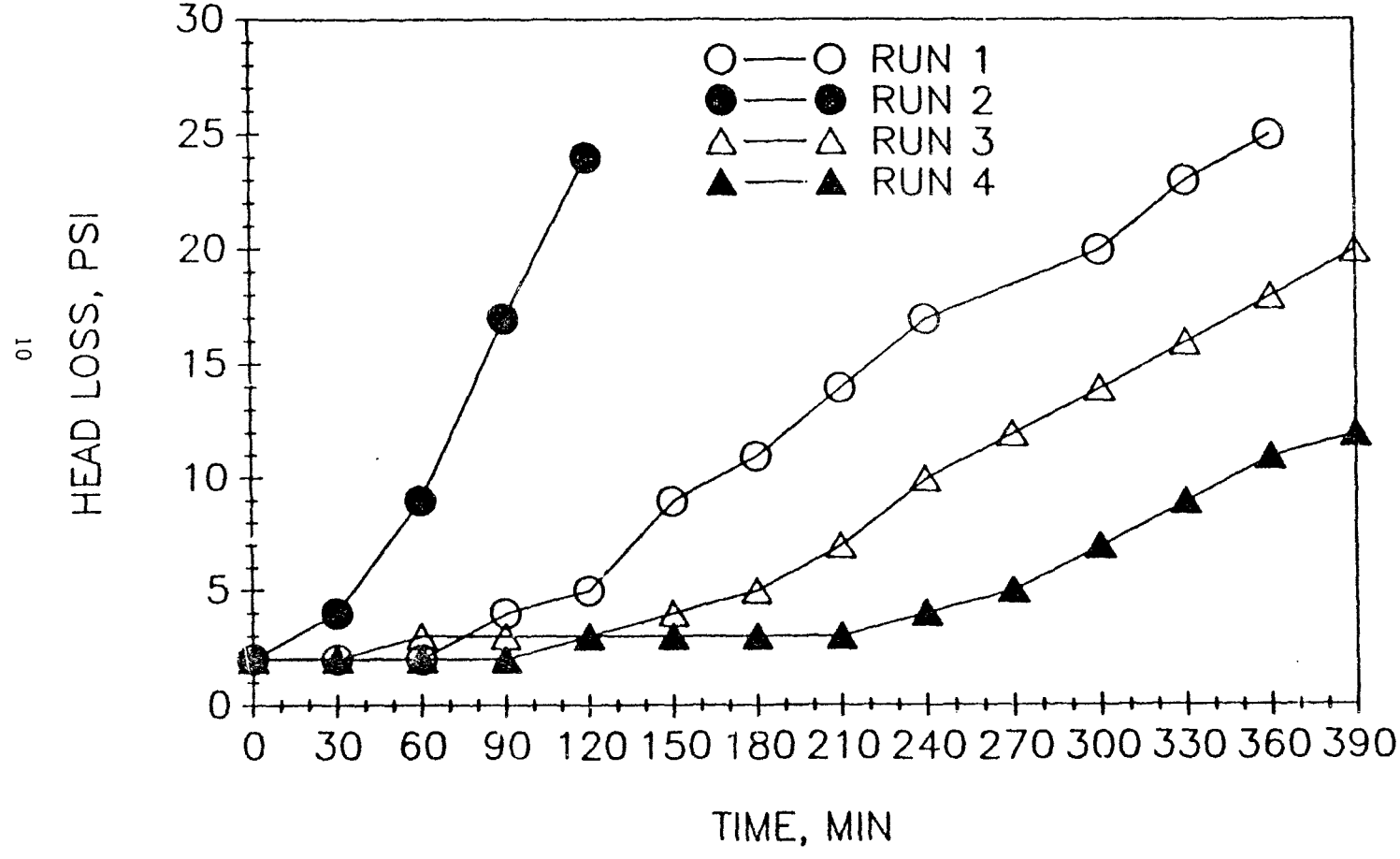


Figure 3. Prefilter performance, 150 mg/L fine test dust added: head loss

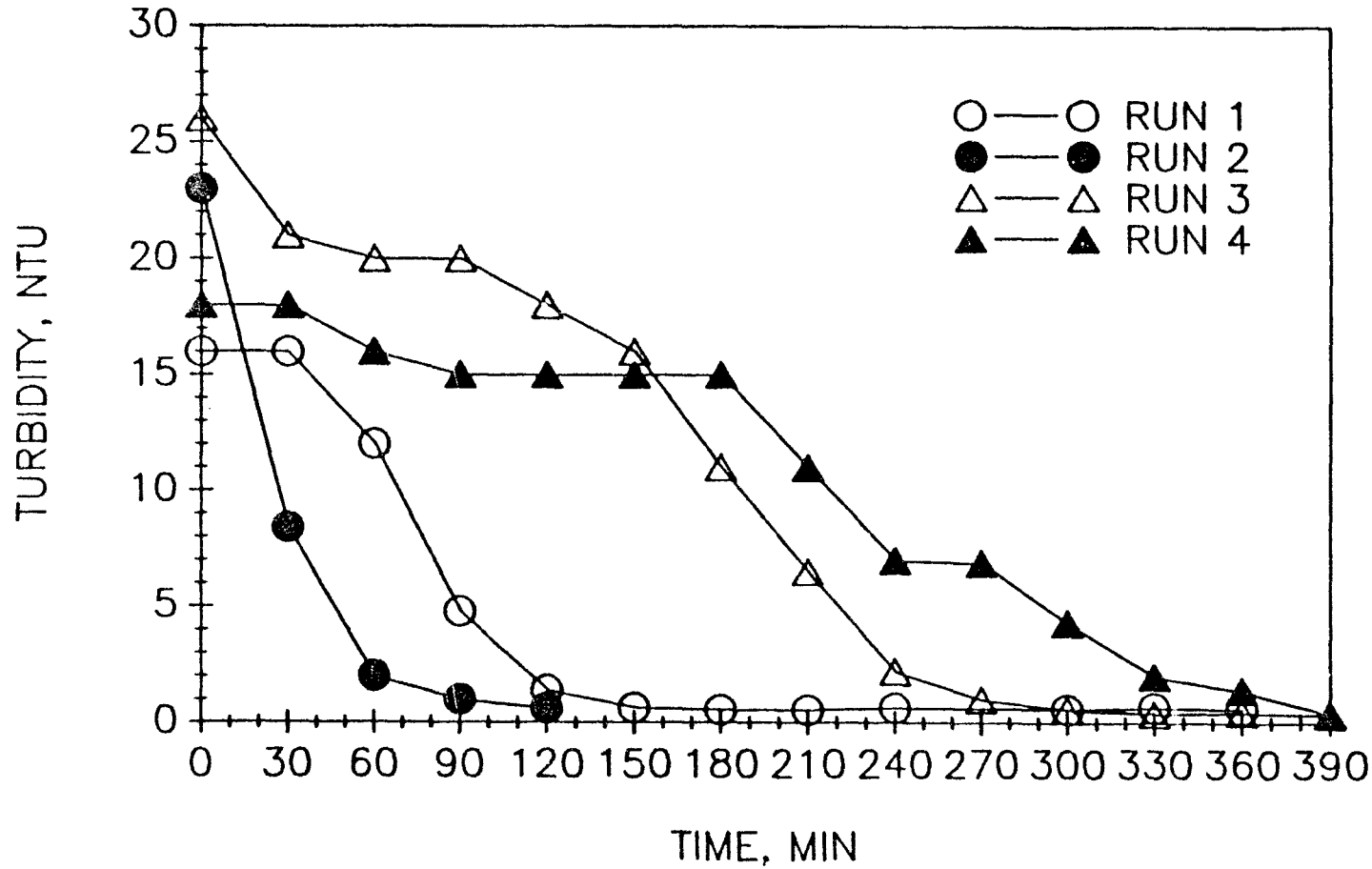


Figure 4. Prefilter performance, 150 mg/L fine test dust added: product turbidity

TABLE 2. LATEX BEAD REMOVAL BY PREFILTER[a]

Time, min	Pressure, psi Inlet	Outlet	Flow Rate gpm	Latex Bead Challenge inoculum	Sample recovery/L	% Total removal
0	34	32	0.92	b		
15	35	32	0.92	b		
30	35	33	0.90	1.82×10^6	1.51×10^5	91.7
60	35	32	0.90	b		
75	34	31	0.90	1.82×10^6	1.36×10^5	92.5
90	35	28	0.90	b		
120	34	25	0.87	1.82×10^6	8.80×10^3	99.5
135	34	20	0.82	b		
150	34	17	0.77	b		
165	34	15	0.71	b		
180	34	12	0.61	b		
195	34	10	0.56	b		
210	34	7	0.50	1.82×10^6	6.78×10^3	99.6

a. Spiral wound cotton, 10 inch.
b. Not sampled.

ROWPU Prefilter Microbial Tests

Tests of the 5.0 μm prefilter were performed using the Guide Standard and Protocol general challenge water,[2] with the addition of AC fine test dust (150 mg/L), K. terrigena (1.0×10^8/L), R. rubra (1.0×10^7/L), and 3.7-μm latex beads (1.0×10^7/L). C. parvum oocysts used in the challenge were diluted into 600 ml sterile water containing red food coloring (105 mL), with or without newborn calf serum (6.0 mL) and 10 percent Tween 20™ (6.0 mL). The oocysts (ca. 2.0×10^6/L) were placed in a stirred flask connected to the challenge water inlet line to the prefilter at the start of the test run; just prior to each sample collection, the cysts were injected into the feedline at a rate of 50 mL/minute with a peristaltic pump. In the first run (Table 3), the filtered samples were collected after a 1-minute injection of cysts. A 32.4 percent loss of cysts in the challenge inoculum was attributed to surface adsorption. To reduce the loss in the second run (Table 4), the flask and feedlines were coated with 1 percent newborn calf serum and 0.1 percent Tween 20™ prior to addition of the cyst suspension. The cyst loss was further reduced in the third run (Table 5) when samples were collected after a 3-minute injection of cysts. Since the injection rate of cysts into the feedline remained constant throughout each run, the numbers of cysts in the challenge inoculum increased as the flow rate decreased. Oocyst and oocyst simulant removals increased as did turbidity removal as the filters clogged and pressure built up across the cartridge. There was significant variation among prefilters as determined by the amount of water filtered before clogging occurred. Comparison of oocyst vs simulant at each sampling time for the 3 runs indicated good agreement with respect to removal efficiency. Removal of

TABLE 3. PREFILTER MICROBIAL REMOVAL: RUN 1

SAMPLE TIME MIN.	FLOW RATE ML/MIN	PRESSURE (PSI) INLET	PRESSURE (PSI) OUTLET	TURBIDITY NTU	CHALLENGE ORGANISM	CHALLENGE INOCULUM/L	SAMPLE RECOVERY/L	% TOTAL REMOVAL
0	3400	36	34	29	not sampled			
30	3400	36	34	13	C. parvum	6.48×10^5	8.00×10^4	87.6
					K. terrigena	1.80×10^8	$\geq 7.75 \times 10^7$	≤ 56.9
					B. subtilis	3.30×10^6	1.40×10^6	57.6
					Accubeads	1.70×10^7	2.54×10^6	85.1
120	3200	36	33	8.2	C. parvum	4.80×10^5	$<1.00 \times 10^3$	>99.8
					K. terrigena	1.73×10^8	5.23×10^7	69.8
					B. subtilis	1.23×10^7	1.77×10^6	85.6
					Accubeads	1.56×10^7	2.25×10^6	85.6
390	1800	36	15	0.7	C. parvum	2.86×10^5	$<1.00 \times 10^3$	>99.6
					K. terrigena	1.67×10^8	1.53×10^6	99.1
					B. subtilis	1.57×10^7	2.50×10^4	99.8
					Accubeads	1.29×10^7	1.07×10^5	99.2

Total gallons filtered = 284

TABLE 4. PREFILTER MICROBIAL REMOVAL: RUN 2

SAMPLE TIME MIN.	FLOW RATE ML/MIN	PRESSURE (PSI) INLET	PRESSURE (PSI) OUTLET	TURBIDITY NTU	CHALLENGE ORGANISM	CHALLENGE INOCULUM/L	SAMPLE RECOVERY/L	% TOTAL REMOVAL
0	3500	34	33	30	not sampled			
30	3500	34	33	18	C. Parvum	6.80×10^5	1.12×10^5	83.5
					K. terrigena	6.30×10^7	2.57×10^7	59.2
					R. rubra	1.03×10^7	5.10×10^6	50.5
					Accubeads	2.16×10^7	3.05×10^6	85.9
120	3200	34	30	2	C. Parvum	1.20×10^6	8.48×10^3	99.3
					K. terrigena	6.30×10^7	7.00×10^6	88.9
					R. rubra	1.13×10^7	3.60×10^5	96.8
					Accubeads	1.54×10^7	6.53×10^5	95.8
240	2400	34	14	0.4	C. Parvum	1.79×10^6	6.72×10^3	99.6
					K. terrigena	6.30×10^7	1.47×10^6	97.7
					R. rubra	1.37×10^7	1.40×10^5	99.0
					Accubeads	1.55×10^7	3.12×10^5	98.0

Total gallons filtered = 196

TABLE 5. PREFILTER MICROBIAL REMOVAL: RUN 3

SAMPLE TIME MIN.	FLOW RATE ML/MIN	PRESSURE (PSI) INLET	PRESSURE (PSI) OUTLET	TURBIDITY NTU	CHALLENGE ORGANISM	CHALLENGE INOCULUM/L	SAMPLE RECOVERY/L	% TOTAL REMOVAL
0	3500	35	33	31	not sampled			
30	3450	35	33	14	C. parvum	1.56×10^6	8.93×10^5	42.8
					K. terrigena	5.37×10^7	9.50×10^6	82.3
					B. rubra	1.20×10^7	5.93×10^6	50.6
					Accubeads	2.44×10^7	1.19×10^7	51.2
120	3200	35	28	0.75	C. parvum	2.55×10^6	1.27×10^4	99.5
					K. terrigena	6.30×10^7	1.17×10^6	98.1
					B. rubra	1.83×10^7	2.97×10^5	98.4
					Accubeads	2.47×10^7	3.84×10^5	98.4
210	1950	35	14	0.2	C. parvum	3.07×10^6	9.40×10^3	99.7
					K. terrigena	7.23×10^7	1.07×10^6	98.5
					B. rubra	1.43×10^7	1.10×10^5	99.2
					Accubeads	1.91×10^7	1.43×10^4	99.9

Total gallons filtered = 168

bacteria by the 5.0-μm prefilter was minor, as was anticipated.

Final Filter Incorporation Study

In these tests, the 3.0-μm filters described above were incorporated in-line after the 5.0-μm prefilters so that the complete bypass approach could be evaluated. Again, the Guide Standard and Protocol was followed.[2] Virus removal capabilities were not evaluated during this study because of the extreme difference in size of the virus versus the filter pore size (ca. 0.02 μm vs 3.0 μm) and lack of active charges on the filter material to effect virus adsorption. The candidate systems each received the microbial challenge in tap water for the first 5 days of operation, followed by up to 5 days (or to the point where irreversible clogging of the filters occurred) in which the filters received the worst-case water containing AC dust, humic acids and high TDS at pH 7.7 or 9.0. The 48-hour stagnation test specified in the Guide Standard and Protocol was not performed, since the proposed ROWPU bypass system is intended for uninterrupted service.

Challenge organisms were K. terrigena bacteria, C. parvum protozoan cysts, and 3.7 μm latex beads; challenge levels were those stated in the Guide Standard and Protocol.[2] The challenge bacteria and latex beads were added to the challenge water reservoir tank and maintained throughout the study. Sample ports were located in the test stand just prior to the 5.0-μm prefilters, after the prefilters, and in the product water reservoir following each 3.0-μm filter. C. parvum oocysts were added through an injection port to the water feedline just after the prefilters. (Note that C. parvum was used as a challenge only to the final filters in order to minimize handling of the pathogenic and chlorine-resistant cysts.) The cysts were introduced and sampled using a pulse chase approach in which the cysts were injected for a period just long enough to maintain plug flow characteristics of the cysts in the system. Calf serum and Tween 20™ were added as before to prevent adsorption of oocysts to the surfaces of the stock container and tubing, and red food coloring was added to provide a visual indication that a steady state in the pulse chase oocyst challenge had occurred before sampling. Samples were taken after red dye had gone completely through the system for ca. 2 minutes. The cyst samples were taken at the sample collection ports in front of and after the final polishing filters.

The test system was initially operated at a constant flow of 3 gpm so that each final filter cartridge saw a flow rate of 1 gpm for both the dechlorinated tap water and the worst-case water. In this mode approximately 750 gal of challenge water was passed through the test system each operational day (250 gal/candidate filter). Daily runs were continued until the differential water pressures from the final polishing filters exceeded 20 psi, which occurred after 1-2 days of operation in worst-case water. Test operations were then concluded with final microbiological sampling. The results are summarized in Tables 6 and 7. These tables show the levels of each organism (averaged over three filters of each type) at each of the three sampling points and the total percent removals for the complete filtration system.

TABLE 6. FINAL FILTER MICROBIAL REMOVAL: RUN 1[a]

Day	Liters(gal)/ Filter	Flow Rate gpm/filter	Pressure psi	Challenge Organisms	Initial Challenge/L	Prefilter Sample/L	Final Filter Sample/L	% Total Removal
				GENERAL WATER				
1	730 (193)	1	11	K. terrigena	1.3×10^8	4.3×10^7	$<1.9 \times 10^6$	>98.60
				Accubeads	9.8×10^6	3.4×10^6	6.5×10^2	99.99
				C. paryum	---	2.0×10^6	$<1.0 \times 10^3$	>99.95
2	920 (243)	1	11	Not Sampled				
3	969 (256)	1	11	K. terrigena	6.3×10^7	8.3×10^6	2.8×10^6	95.55
				Accubeads	1.1×10^7	5.0×10^5	$<1.0 \times 10^3$	>99.99
				C. paryum	---	2.8×10^6	$<1.0 \times 10^3$	>99.96
4	935 (248)	1	12	Not Sampled				
5	939 (248)	1	12	K. terrigena	6.9×10^7	7.3×10^6	2.4×10^6	96.19
				Accubeads	1.4×10^7	3.0×10^5	$<1.0 \times 10^3$	>99.99
				C. paryum	---	2.3×10^6	$<1.0 \times 10^3$	>99.96
				WORST CASE WATER				
6	920 (243)	1	14	K. terrigena	2.8×10^8	8.0×10^7	2.8×10^7	90.00
				Accubeads	7.3×10^6	1.8×10^6	4.4×10^2	99.99
				C. paryum	---	9.5×10^5	$<1.0 \times 10^3$	>99.89
7	641 (170)	1	27	K. terrigena	1.5×10^8	8.0×10^7	7.0×10^5	99.53
				Accubeads	1.0×10^7	6.4×10^6	$<1.0 \times 10^3$	>99.99
				C. paryum	---	2.2×10^6	$<1.0 \times 10^3$	>99.95

a. Polypropylene depth filter

TABLE 7. FINAL FILTER MICROBIAL REMOVAL: RUN 2[a]

Day	Liters(gal)/ Filter	Flow Rate gpm/Filter	Pressure psi	Challenge Organisms	Initial Challenge/L	Prefilter Sample/L	Final Filter Sample/L	% Total Removal
				GENERAL WATER				
1	922 (244)	1	9	K. terrigena	1.1×10^8	1.4×10^7	5.4×10^6	95.09
				Accubeads	1.1×10^7	9.5×10^5	1.4×10^4	99.87
				C. parvum	---	2.0×10^6	8.9×10^4	95.55
2	953 (252)	1	9	Not Sampled				
3	896 (237)	1	9	K. terrigena	9.1×10^7	3.5×10^6	6.7×10^5	99.26
				Accubeads	1.1×10^7	2.8×10^5	1.0×10^3	99.99
				C. parvum	---	2.6×10^6	2.1×10^4	99.19
4	1033 (273)	1	10	Not Sampled				
5	959 (253)	1	11	K. terrigena	8.1×10^7	2.7×10^6	9.3×10^5	98.85
				Accubeads	1.2×10^7	1.5×10^5	1.0×10^3	99.99
				C. parvum	---	3.1×10^6	2.7×10^4	99.13
				WORST CASE WATER				
6	378 (100)	1	27	K. terrigena	8.3×10^7	3.0×10^7	5.0×10^6	93.97
				Accubeads	8.3×10^6	6.5×10^6	5.9×10^5	92.89
				C. parvum	---	2.2×10^6	1.9×10^5	91.36

a. Pleated polypropylene filter

From Table 6 it is evident that 90-98 percent removal of K. terrigena bacteria was achieved in the total bypass system using polypropylene depth filters, well below the U.S. Environmental Protection Agency (USEPA) criterion of 99.9999 percent removal. On the other hand, removal of latex beads generally exceeded 99.99 percent (except for day 6), well above the USEPA criterion of 99.9 percent removal. The final filters alone removed C. parvum cysts with greater than 99.9 percent efficiency throughout the tests. No important loss of filterability was observed for either cysts or beads in worst-case water vs the dechlorinated tap water. Bacterial removals for pleated polypropylene filters were comparable with those for the polypropylene depth filters, but the pleated filters were much less effective in removal of beads and cysts and did not consistently meet USEPA criteria for any challenge (Table 7).

A separate test was conducted to evaluate the polypropylene depth filters at a flow rate of 2 gpm (Table 8). For this study two filters were used in parallel instead of three; the prefilter system was maintained as before. Sampling for cysts and beads in the dechlorinated tap water challenge was performed after throughputs of 200 gal and 1200 gal per filter. The worst-case water challenge was sampled only after differential pressure exceeded 25 psi (a value concurred in by the manufacturer[4]). Bacterial removals were not evaluated. The only other significant departure from the protocol was that daily filtration was not discontinued after 250 gal of water, but was continued throughout the day. The higher flow rate did not adversely affect the filtration of cysts or their simulants in either the dechlorinated tap water or, upon clogging, in the worst-case water. A comparison of Table 8 with Table 6 indicates that total removal percentages were similar. During this run the flow rate was dropped to 1 gpm/filter for a short period of time to directly compare bead removals for the same filter at the different flow rates; the removal efficiencies were comparable. Worst-case water also had no significant impact on the efficiency of removal of the cyst size particles, and a test performed on a separate set of polypropylene depth filters at pH 9 in worst-case water demonstrated removal efficiencies for cysts and beads essentially identical to those observed at pH 7.7 (Table 8).

CONCLUSIONS

Two different filter combinations have been tested as candidate systems for bypassing the reverse osmosis membranes of the Army's ROWPU when treating fresh water, namely, a spiral-wound cotton prefilter of 5.0-μm nominal pore size combined with a melt-blown polypropylene depth filter of 3.0-μm absolute pore size and the same prefilter combined with a pleated polypropylene filter of 3.0-μm absolute pore size. Test organisms were Klebsiella terrigena (a representative enteric bacterium), Cryptosporidium parvum (an enteric protozoan pathogen) oocysts, Rhodotorula rubra (a yeast, used to test prefilters only) and 3.7-μm latex beads; challenge waters were dechlorinated tap water and a worst-case water containing AC fine test dust and humic acid. Physical removal of C. parvum oocysts appeared to correlate well with removal of R. rubra and latex beads.

TABLE 8. FINAL FILTER MICROBIAL REMOVAL: RUN 3[a]

Day	Liters(gal)/ Filter	Flow Rate gpm/Filter	Pressure psi	Challenge Organisms	Initial Challenge/L	Prefilter Sample/L	Final Filter Sample/L	% Total Removal
				GENERAL WATER				
1	946 (250)	2	19	Accubeads	1.23×10^7	1.59×10^6	2.20×10^2	99.99
				C. parvum	---	2.76×10^6	$<1.00 \times 10^3$	>99.96
1	1135 (300)	1	8	Accubeads	1.23×10^7	1.59×10^6	4.76×10^3	99.96
				C. parvum	Not Challenged			
2	3407 (900)	2	23	Accubeads	1.56×10^7	1.52×10^6	$<1.00 \times 10^3$	>99.99
				C. parvum	---	2.13×10^6	$<1.00 \times 10^3$	>99.95
				HORST CASE WATER (pH 7.7)				
3	394 (104)	2	26	Accubeads	1.45×10^7	4.52×10^6	$<1.00 \times 10^3$	>99.99
				C. parvum	---	1.61×10^6	$<1.00 \times 10^3$	>99.94
				GENERAL WATER				
1-2	3179 (840)	2	21.5	Accubeads	Not Counted	2.09×10^6	*[b]	
				C. parvum	---	2.73×10^6	*	
				HORST CASE WATER (pH 9.0;TEMP 9°C)				
3	144 (38)	2	27	Accubeads	1.82×10^7	9.58×10^6	5.60×10^2	99.99
				C. parvum	---	1.63×10^6	$<1.00 \times 10^3$	>99.94

a. Polypropylene depth filter
b. Results invalid; discarded

Neither the prefilters nor the reverse osmosis bypass filters were adequate for the removal of K. terrigena; the 3.0-micron filters at best could reduce the bacterial challenge by no more than 2 orders of magnitude. (As anticipated, improved reductions for all microbial challenges were achieved with time in worst-case water, corresponding to reduction in average pore size of the filter material through particle entrapment.)

The polypropylene depth filter, tested separately, achieved better than 99.9 percent reduction of C. parvum oocysts (the USEPA criterion) at filtration rates of 1-2 gpm, whether challenged with dechlorinated tap water or worst-case water (pH 7.7-9.0). Latex bead removal for the complete system generally exceeded 99.99 percent. The pleated polypropylene final filter, on the other hand, did not achieve 99.9 percent reduction of C. parvum oocysts at a filtration rate of 1 gpm in either challenge water, and removal of latex beads for the complete system was irregular and inconsistent with the putative absolute pore size.

The data from this study would support the use of filtration of drinking water for the removal of protozoan cysts even down to the size range of C. parvum oocysts of ca. 4-μm diameter. Based upon the analysis of the complete reverse osmosis bypass system data, it is concluded that a system using polypropylene depth filters of 3.0-μm absolute pore size would be capable of providing adequate removal of the cyst challenges throughout their effective use life. Although this system appears to be a suitable candidate for the RO bypass of Air Force and Marine Corps ROWPUs, pre- or post-disinfection (at normal field military concentrations) would still be required to remove residual bacteria and enteric viruses from the product water.

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APPENDIX A

PERFORMANCE OF PREFILTER: 150 MG/L AC FINE TEST DUST ADDED

TABLE A1. RUN NO. 1

Time min	Flow gpm	Inlet pressure psig	Outlet pressure psig	Product turbidity NTU
0	0.85	34	32	16
30	0.82	34	32	16
60	0.82	34	32	12
90	0.71	34	30	4.8
120	0.74	34	29	1.4
150	0.69	34	25	0.64
180	0.66	34	23	0.55
210	0.61	34	20	0.55
240	0.56	34	17	0.65
300	0.53	34	14	0.60
330	0.48	34	11	0.67
360	0.45	34	9	0.60

TABLE A2. RUN NO. 2

Time min	Flow gpm	Inlet pressure psig	Outlet pressure psig	Product turbidity NTU
0	0.90	34	32	23
30	0.87	34	30	8.4
60	0.79	34	25	2.0
90	0.63	34	17	1.0
120	0.53	34	12	0.62

TABLE A3. RUN NO. 3

Time min	Flow gpm	Inlet pressure psig	Outlet pressure psig	Product turbidity NTU
0	0.85	34	32	26
30	0.82	34	32	21
60	0.77	34	31	20
90	0.77	34	31	20
120	0.74	34	31	18
150	0.74	34	30	16
180	0.74	34	29	11
210	0.66	34	27	6.5
240	0.63	34	24	2.2
270	0.61	34	22	1.0
300	0.58	34	20	0.57
330	0.53	34	18	0.40
360	0.50	34	16	0.45
390	0.50	34	14	0.35

TABLE A4. RUN NO. 4

Time min	Flow gpm	Inlet pressure psig	Outlet pressure psig	Product turbidity NTU
0	0.85	35	33	18
30	0.69	35	33	18
60	0.69	35	33	16
90	0.66	35	33	15
120	0.63	35	32	15
150	0.63	35	32	15
180	0.63	35	32	15
210	0.63	35	32	11
240	0.63	35	31	7
270	0.59	35	30	6.9
300	0.58	35	28	4.3
330	0.58	35	26	2.0
360	0.56	35	24	1.4
390	0.56	35	23	0.4

APPENDIX B

GLOSSARY OF TERMS

cfu	colony forming units
gal	gallon(s)
gph	gallons per hour
gpm	gallons per minute
NCEL	Naval Civil Engineering Laboratory
NTU	nephelometric turbidity units
P&ID	process and instrumentation diagram
PBS	phosphate buffered saline
psi	pounds per square inch
psig	pounds per square inch (gauge)
PVC	polyvinyl chloride
RO	reverse osmosis
ROWPU	reverse osmosis water purification unit
SSTI	Separation Systems Technology, Inc.
TDS	total dissolved solids
TOC	total organic carbon
USABRDL	U.S. Army Biomedical Research and Development Laboratory
USEPA	U.S. Environmental Protection Agency

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